

IN THE SPECIFICATION

Amend the paragraph beginning on page 4 at line 14 as follows:

According to the present invention, the biosensor is formed by screen printing and includes an electrode layer (electrode area) comprising two or three electrodes, which are a working electrode, a reference electrode and an auxiliary electrode (tri-electrode) on an insulating substrate. An active reaction layer containing reactant, reaction catalyst, mediator, wetting agent and surfactant is spread on the surface of the electrode layer. A sample inflow channel ~~area~~ above the electrodes between an upper cover and a middle insulating layer is used to introduce sample solution into the electrode area and the active reaction layer by siphon or capillary. Ingredient of the sample can be analyzed by electrochemical potentiometric or amperometric method. Further, the present invention provides an upwardly extended close ~~space~~ chamber formed within the upper cover above the electrode area adjacent to the front of conductive wires, which can be effectively used to control sample volume and “fill-and-position” the sample.

Amend the paragraph beginning on page 4 at line 29 as follows:

Fig. 1 is an exploded view illustrating the structure of an electrode sensor by screen printing according to the present invention with a ~~U-shaped-opening~~ slot;

Fig. 2 is an exploded view illustrating the structure of an electrode sensor by screen printing according to the present invention with a T-shaped ~~openings~~ slot;

Fig. 3 is a longitudinal, cross-sectional view of the electrode sensor by screen printing according to the present invention;

Fig. 4 is an exploded view illustrating the structure of an upper cover with an upwardly extended closed ~~space~~ chamber formed therein according to the present invention;

Fig. 5 is a longitudinal, cross-sectional view of the structure of ~~an~~ the upper cover with an upwardly extended closed space chamber formed therein according to the present invention;

Fig. 6 is a longitudinal, cross-sectional view of the structure of an electrode sensor with an upwardly extended closed space chamber formed therein; and

Fig. 7 shows the influence of whole blood volume on measurements.

Amend the paragraph beginning on page 5 at line 16 as follows:

According to the present invention, the structure of a tri-electrode biosensor 10 by screen printing is illustrated in Fig. 1. Conductive wires 12 made of electrically conductive gel such as silver and gold, are formed on an insulating ~~base plate~~ substrate 11, which is made of polyvinylchloride (PVC), polyester (PE), polyether, polycarbonate, or the like, by screen printing. Electrode strips are then formed on top of the conductive wires 12 by printing another layer of electrically conductive materials such as carbon, gold, and platinum. Electrodes containing a working electrode U1U3, a reference electrode U1U4 and an auxiliary electrode 15 (no auxiliary electrode in a bi-electrode sensor) are ~~formed~~ disposed at one end above the layer of conductive wires 12. The corresponding contact ports 13', 14' and 15' at the other end with respect to the electrodes 13, 14, 15 can be connected to a measuring device (not shown in figure) and a device activation line 16 can be automatically recognized by the measuring device. A non-electrically conductive or an middle insulating ~~middle~~ layer 17, which acts as an insulating dielectric layer as well as provides spacing with a U-shaped openings slot 17a formed therein, is ~~formed~~ disposed above the insulating ~~base plate~~ substrate 11 containing electrodes by adhesion or screen printing. ~~Channel Slot 17a~~ Channel Slot 17a designates a sample inflow channel area and an upwardly extended closed space chamber 18a with a volume of about $2\ \mu\text{l}$, is formed within an upper cover 18 ~~opposing to~~ above and in communication with one end of the inflow channel area. An active reaction layer 20 containing substances of reactant, reaction catalyst (such as enzyme), mediator (such as directly ferrocene, tetrathiofulvalene), wetting agent (cellulose, hydroxyethyl cellulose, carboxymethyl, cellulose, polyvinyl alcohol, polyvinyl, pyrrolidone and gelatine, etc.), and surfactant (Tween 20, triton X-100, surfynol, mega 8, etc.) is spread on the surface of the electrodes 13, 14, 15, which defines an electrode reaction area ~~where reactions take place~~.

When the upper cover 18 is adhered to the middle layer 17, the slot 17a defines ~~The a~~ capillary inflow channel-7a, which allows sample such as blood to be rapidly introduced into and filled the electrode reaction area by capillary upon contact with ~~the front tip thereof~~ its front tip, ~~is formed when the upper cover 8 is adhered to the middle layer 7.~~ Reactions induced by reaction catalyst can subsequently take place between reactant and mediator, in which electric current can be generated and measured by the measuring device. The inflow channel can provide the electrodes with rapid fill-in time (less than 1 second) and a minute amount of sample (less than 1 μ l).

Amend the paragraph beginning on page 6 at line 19 as follows:

The structure of another electrochemical tri-electrode sensor 10 according to the present invention is illustrated in Fig. 2. Conductive wires 12 of electrically conductive materials such as silver, silver chloride, and gold, are formed on an insulating ~~base plate~~ substrate 11, by screen printing. Electrodes of electrically conductive materials such as carbon, carbon, and platinum, comprising a working electrode 13, a reference electrode 14 and an auxiliary electrode 15 are printed on the conductive wires 12. The corresponding electrodes 13', 14' and 15' with respect to the electrodes 13, 14, 15 are contact ports to a measuring device (not shown in figure), whereas a device activation line U16 can be automatically recognized by the measuring device. A spacing middle layer 17 of insulating material with a T-shaped opening slot 17a formed there, is formed on top of the insulating ~~base plate~~ substrate 11 containing electrodes by adhesion or coating a layer of insulating paste by screen printing. An upper cover 18 containing an upwardly extending closed space chamber 18a with volume of about 2 μ l is ~~added~~ formed on top of the spacing middle layer 17 and the closed space chamber 18a is positioned above the intersection of the T-shaped opening slot 17a. A sample inflow channelled 7a is formed between the spacing middle layer 17 and the upper cover 18 while 17b and 17c form air vents on the opposite sides of the sensor 10. Sample such as blood can be rapidly introduced into and field an electrode reaction are 20 by capillary upon contact with the front tip of capillary inflow channel-7a. Similar to Fig. 1, ~~the design of the front edge of the sample is configured not to go beyond the front tip of~~ chamber 18a along the

inflow channel. In addition, same venting effect can be achieved by removing either air vent 17b or 17c.

Please replace the heading on page 7 at line 8 with the following replacement heading:

Insulating substrate

Amend the paragraph beginning on page 7 at line 9 as follows:

Insulating ~~base plate~~ substrate †can be made of a variety of materials such as polymer, plastics, and ceramics. Materials should be chosen according to the requirement and application of electrode materials. For example, soft material should be chosen for invasive type sensors to reduce pain and avoid hurting tissues. For such sensors, insulating polymer materials such as polycarbonate, polyester, polyethylene terephthalate (PET), polyvinylchloride (PVC), polyether, polyamide, polyurethane, polyamide, etc., can be adapted. On the other hand, rigid materials which are not easy to be ruptured or bent, such as ceramics including silica or aluminum dioxide, can be adapted. With regard to measurement outside a human body, width of the insulating ~~base plate~~ substrate is generally between 3 and 15 μm and more precisely between 5 and 10 μm . Thickness is between about 50 and 800 μm and more precisely between 200 and 400 μm . Length of the insulating ~~base plate~~ substrate depends on different factors and may be between about 1 and 8 cm and more precisely between 2 and 5 cm.

Amend the paragraph beginning on page 7 at line 24 as follows:

As illustrated in Fig. 1, a layer of electrically conductive wires 12 made of electrically conductive materials such as silver, gold and platinum, is formed by screen printing, which is for connecting electrodes and a measuring device. Materials with high electrical conductivity and low resistance can reduce impedance of the electrodes and therefore increase signals of

detected current. Electrically conductive material such as carbon paste can be printed on top of the wires 12 and a device activation line 16 can be automatically recognized by the measuring device. Apart from a reference electrode 14, wires 12 are completely coated. The exposed surface of silver wire in electrode 14 can be processed electrochemically to form a reference electrode of silver chloride, or processed electrochemically to form a reference electrode of silver chloride, or printed by silver/silver chloride ink. In the latter case, silver chloride processing is not necessary.

Please replace the heading on page 8 at line 5 with the following replacement heading:

Middle insulating layer

Amend the paragraph beginning on page 8 at line 6 as follows:

Middle insulating middle layer 17 can be formed by printing or adhering dielectric material above electrodes, which in turn covers the carbon surface not required to be exposed and provides a reaction region with fixed area.

Amend the paragraph beginning on page 8 at line 10 as follows:

Reaction reagents are spread on top of electrodes, which include reaction catalyst, buffer solution, binder, mediator, surfactant, etc. For example, when glucose is measured, the catalyst can be glucose oxidase or dehydrogenase. The ingredient of binder contains polymer or wetting agent including cellulose, polyvinyl alcohol, gelatine, surfactant, etc., such as Tween-20, Triton X-100, Surfydol, and Mega 8, which can dissolve and disperse sample and reagents and provide hydrophile and dispersion for capillary inflow channel area. Therefore, the reaction reagent layer can provide both reaction and capillary, which not only fills sample in electrodes for analysis of reactions, but also provides electric current generated by reactions in electrodes for quantitative analysis of the sample. Preferred mediator, depending on requirement of different measurements, should have redox potential between -100 and

+500 mV. For example, ferrocene such as dimethylferrocene, tetrathiafulvalene and derivative or complex of both can be applied. A lower potential can avoid interfering materials in the sample, while higher electron conducting efficiency can provide stronger electric current signals. Buffer solution can maintain pH within a fixed range, generally between 4 and 9 and preferably between 5 and 8. Useable buffer solutions include phosphoric acid, acetate salt, citrate salt, etc., and concentration can range between 10 and 1000 mmole/l and preferably between 30 and 1000 mmole/l.

Amend the heading beginning on page 9 at line 1 as follows:

Capillary inflow ~~layer~~ channel is formed by adding a ~~spacing~~ middle layer 17 and an upper cover 18 on the top of electrodes 13, 14, 15, 17a represents a sample capillary channel and 17b and 17c, which can exist independently, are air vents on opposite side of a sensor 10 (T-shaped design). The volume of the inflow channel ~~area~~ can be adjusted by varying thickness of the ~~spacing~~ middle layer 17 and width of channel 17a. The thickness of the inflow channel ~~area~~ is generally between 20 and 400 μm and preferably between 50 and 200 μm . The length of the hollow inflow channel ~~area~~ is between 2 and 8 mm and the width of which is between 0.5 and 5 mm and preferably between 1 and 2 mm. The volume of the hollow inflow channel ~~area~~ is between 0.05 and 16 μl and a volume between about 0.5 and 4 μl is required when actual measurement is performed. The time between a sample being in contact with the edge of the inflow channel ~~area~~ and filled filling-in the inflow channel ~~area~~ is less than 1 second.

Amend the paragraph beginning on page 9 at line 14 as follows:

The closed protrusion 18a, which defines the chamber in the upper cover 18, can be round, rectangular or of other geometry shape and the desired size can be between 0.5 and 4 mm. The location of an opening of the enclosed protrusion 18a is above the inflow channel and behind a the working electrode 13. Blood sample can be filled in ~~a~~ the reaction area, which the flowing of the sample is then stopped at some point ~~by the opening~~ due to air pressure in the chamber 18a. The ~~spacing~~ middle layer 17 and the upper cover 18 can be made of transparent opaque insulating materials such as plastics or polymers including PVC, Mylar,

etc. ~~Area~~ The protrusion 18a may be transparent for better inspection of sample flowing in by eyes and protection of sensor. The upper cover 18 can be formed by 2 steps. The first step is to form an opening 8a in the upper cover 18, as shown in Fig. 1 and the second step is to apply another thin plate 19 (as shown in Figs. 4 and 5). Figs. 3 and 6 show the sensor illustrated in Fig. 1 in longitudinal, cross-sectional view, which contains the thin plate 19.

Amend the paragraph beginning on page 9 at line 28 as follows:

Filling detecting device is designed to detect if a sample is filled above three electrodes. For a tri-electrode type sensor, if working electrode is disposed at the outer edge of inflow channel area, filling detection can be arranged by using working electrode and auxiliary electrode and by monitoring electric current, potential and impedance. Impedance between working and reference electrodes is infinite by potentiometry when no sample is present and decreases significantly when sample is filled inside the inflow channel area, by which parameter of electrochemical analysis is activated when sample is filled. For a bi-electrode type sensor, similar method can be applied. In order to apply electrodes for filling detection, distribution of electrodes should be the same as direction of sample flow. That is, working electrode needs to be in contact with sample ahead of auxiliary electrode and subsequently complete filling of sample can be determined. Similarly auxiliary electrode can be arranged to be in contact with sample ahead of working electrode, and vice versa.

Amend the paragraph beginning on page 10 at line 28 as follows:

A layer of electrically conductive silver paste is formed on a polypropylene synthetic substrate 11 by 300 mesh screen printing, which is dried and heated for 30 minutes at 50°C, and three electrodes (working electrode 13, reference electrode 14 and auxiliary electrode 15) are printed by carbon paste thereon. The substrate 11 is again heated for 15 minutes at 90°C and printed by insulating gel, which is subsequently dried and hardened under ultraviolet light to form an insulating layer with an inflow reaction are 17c, 17b and 17c (for sensors with air vents). Reaction reagents of 2-6 μ l, containing 0.5-3 units of glucose oxidase, 0.1-1% of polyvinyl alcohol, pH 4.0-9.0 and 10-100 mM potassium phosphate as buffer solution, 10-100 mN potassium chloride, 0.05-0.5% of dimethylferrocene, 0.005%-0.2% tween -20,

0.005%-0.2% of sufynol and 0.1%-1.0% of carboxymethyl cellulose are spread on the recessed inflow channel area 17a. The substrate is dried at 45°C for one hour and an upper cover 18 with an opening 18a formed therein is adhered on top of the substrate 11. A transparent upper cover 19 is pressed above the substrate 11 and sensors can be cut by die cutting from the substrate 11.

Amend the paragraph beginning on page 12 at line 9 as follows:

The method of measurements is to provide whole blood samples with different volume and supply samples by siphon under conditions set out in Example 2. As shown in Fig. 7, when the volume of a sample is insufficient (e.g., of less than 0.5 l), the concentration of glucose is low. Conversely, when the volume of a sample is above 0.8 l, the measured glucose concentration is near that in the sample solution, and the whole amount of the sample cannot be introduced into the sensor. That is, the more the volume of a sample is supplied, the more volume of the sample will be redundant, since inflow reaction channel area is saturated with the sample and cannot accommodate more solution. The front edge of sample ~~is~~ does not go beyond the intersection between ~~8b~~ 18a and the inflow channel area, which is the evidence that the volume of sample solution can be effectively controlled and restricted.